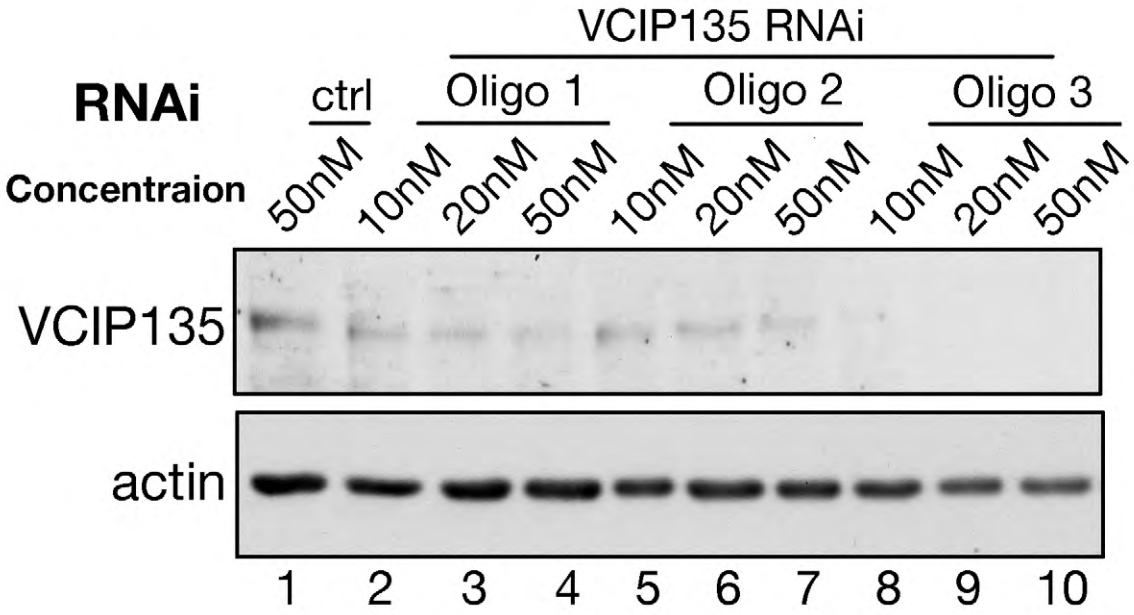

Fig. S1. VCIP135 depletion by three sets of RNAi oligos. HeLa cells were transfected with indicated concentrations of control (ctrl) and VCIP135 RNAi oligos and analyzed by Western blot with the indicated antibodies. Note that Oligo 3 is the most efficient.

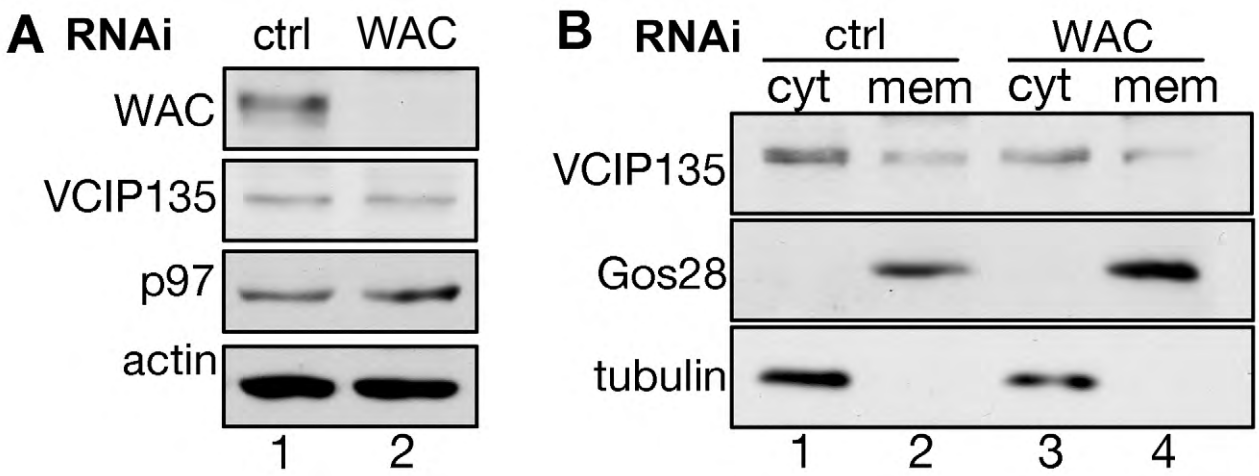

Fig. S2. WAC depletion does not affect VCIP135 membrane association. (A) WAC depletion is efficient. HeLa cells transfected with control or WAC RNAi were subjected to Western blot with indicated antibodies. (B) WAC depletion does not affect VCIP135 membrane association. HeLa cells transfected with indicated RNAi were subjected to subcellular fractionation to separate cytosol (cyt) and membranes (mem) and analyzed by western blot with indicated antibodies.

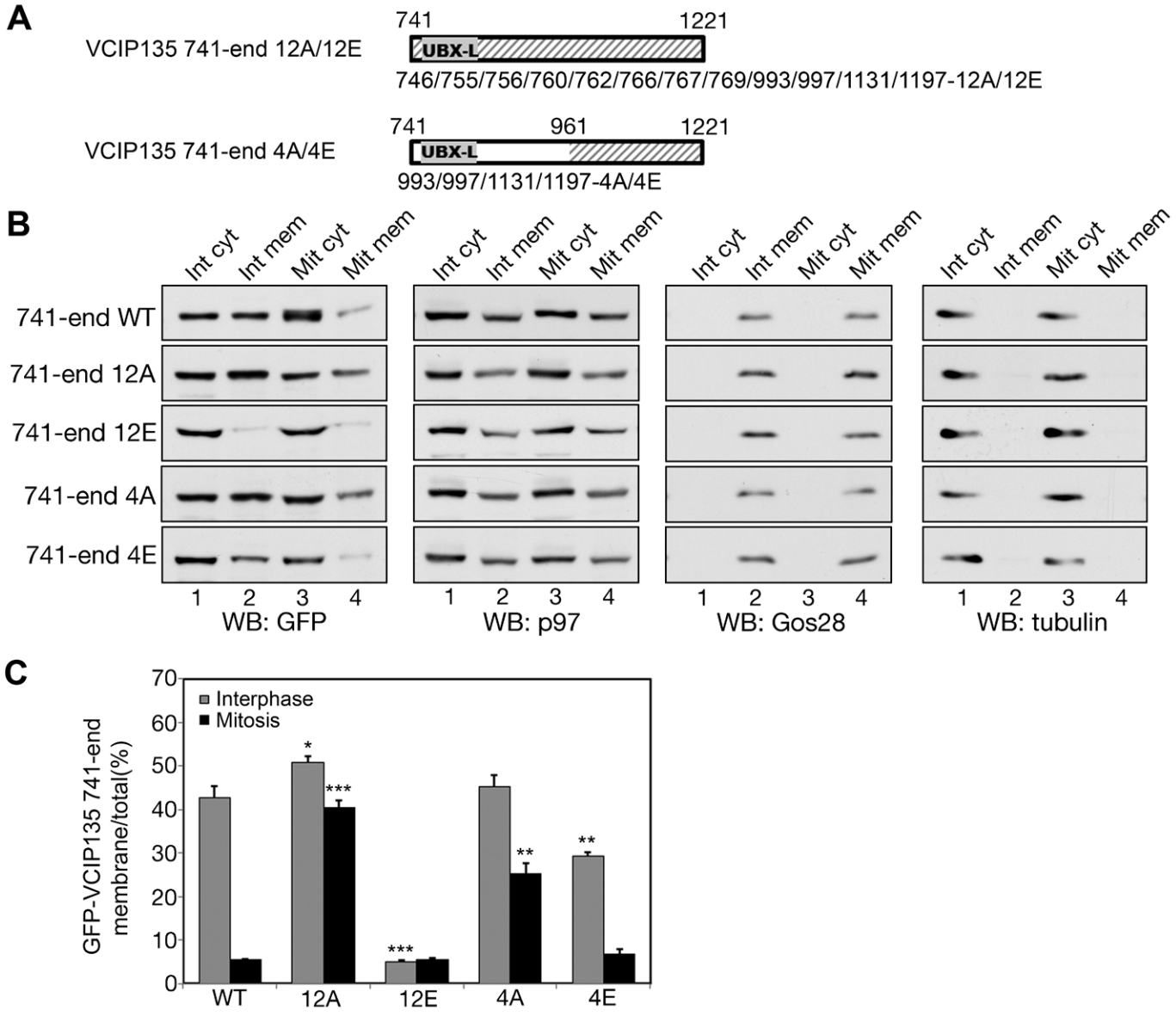

Fig. S3. Phosphorylation of VCIP135 at the C-terminus reduces its membrane association. (A) Schematic representation of the C-terminus of VCIP135 (741-end) non-phosphorylatable mutants (4A or 12A, phosphorylation sites were mutated to alanines) and phosphomimetic mutants (4E and 12E, mutated to glutamic acids). The hatched shading in the diagram shows the region with the indicated phosphorylation sites. (B) Phosphomimetic mutants of the C-terminus of VCIP135 have reduced membrane association. HeLa cells transfected with indicated VCIP135 constructs were synchronized, subcellular fractionated and analyzed by western blot. WT, wild type. Note that the 12A mutant of VCIP135 aa741-end has comparable membrane association between interphase and mitosis, while 12E does not bind to membranes. (C) Quantification of B to show membrane association of indicated WT aa741-end or its mutants in interphase and mitosis from three independent experiments. *P*-value here shows the significant difference of membrane association between wild type and different mutants.

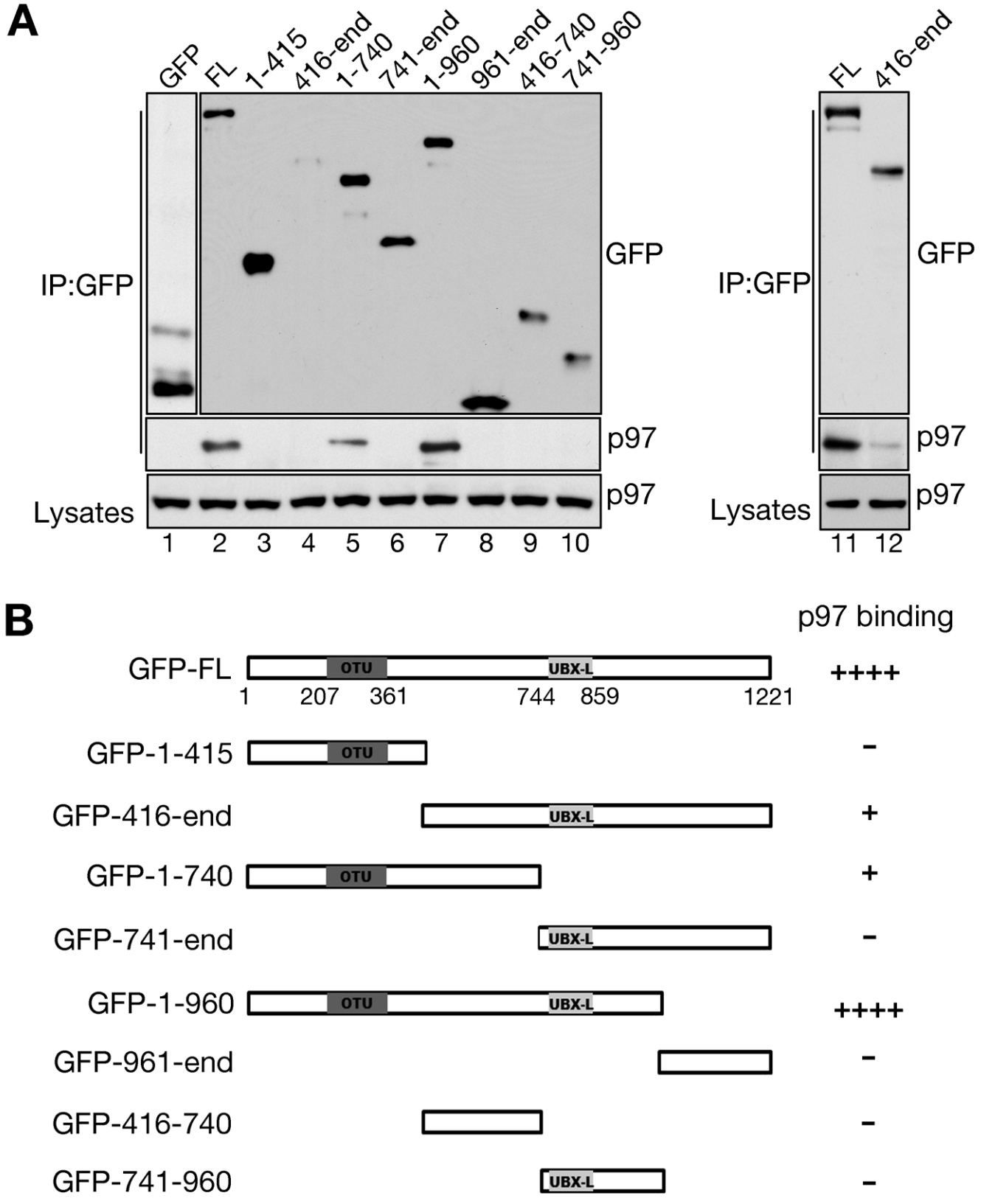

Fig. S4. Mapping the p97-binding sites on VCIP135. HeLa cells transfected with GFP or indicated GFP-tagged VCIP135 full length or truncated mutants were lysed, GFP or VCIP135-GFP was immunoprecipitated (IP) using a GFP antibody followed by Western blot for GFP and p97. FL, full length VCIP135. Summary of VCIP135 constructs and their binding to p97. Each construct was tagged with GFP at the C-terminus. The catalytic domain (OTU) is shaded dark gray and the UBX-like (UBX-L) domain is in light grey. Note that both of the N-terminus of VCIP135 (1-740) and UBX-L domain are required for VCIP135 interaction with p97.

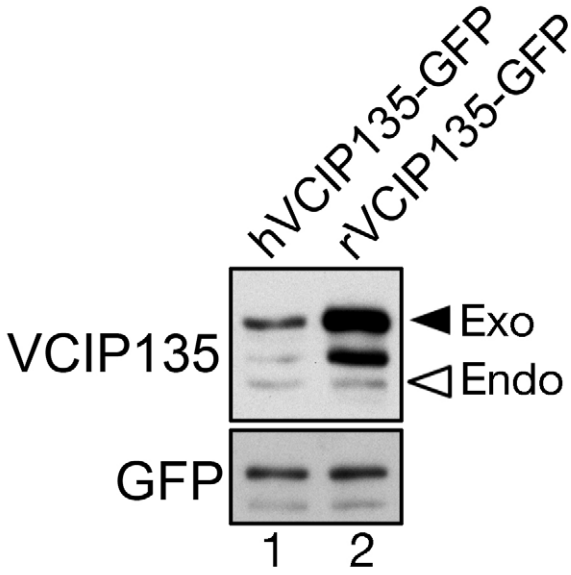

Fig. S5. The VCIP135 antibody has a higher immunoreactivity with rat VCIP135 than its human homologue. HeLa cells transfected with GFP-tagged rat VCIP135 (rVCIP135-GFP) or its human homologue (hVCIP135-GFP, also called VCPIP1) were lysed and analyzed by Western blot with indicated GFP and VCIP135 antibodies. Note that the signal for exogenous (Exo) hVCIP135-GFP is lower than rVCIP135-GFP (lane 1 vs 2), although the GFP and endogenous (Endo) VCIP135 signals in the two lanes are about equal.